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16. ABSTRACT

Purified DNA from the nuclear polyhedrosis viruses of Autographa californica (AcM NPV) and Rachiplusia ou (RoM NPV) were found to be infectious in TN-368 cells employing the calcium phosphate precipitation technique (F.L. Graham and A.J. van der Eb, Virology, 52, 456-467, 1973). Transfection with AcMNPV produced 3600 PFU/µg DNA compared to 2900 PFU/µg DNA with RoMNPV. Of eight baculovirus DNAs tested, only AcMNPV DNA and RoMNPV DNA could transfect TN-368 cells. The in vitro host range of AcMNPV DNA was determingd to be the same as AcMNPV extracellular virus. AcMNPV Form 1 DNA was fourfold more infectious in TN-368 cells than Form II DNA.

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ERRATUM

Volume 101, No. 1 (1980), in the article "Transfection with Baculovirus DNA," by John P. Burand, Max D. Summers, and Gale E. Smith, pp. 286–290: p. 288, column 1, line 19 should read ". . . without the addition of $CaCl_2$ were unsuccessful." Figure 3 legend (A) should read "Ac*M*NPV DNA from the band of heavier density was frozen and thawed twice to produce Form III DNA. . . ."

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Journal Article

Transfection with Baculovirus DNA

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Purified DNA from the nuclear polyhedrosis viruses of *Autographa californica* (Ac*M* NPV) and *Rachiplusia ou* (Ro*M* NPV) were found to be infectious in TN-368 cells employing the calcium phosphate precipitation technique (F. L. Graham and A. J. van der Eb, *Virology*, **52**, 456–467, 1973). Transfection with Ac*M* NPV produced 3600 PFU/μg DNA compared to 2900 PFU/μg DNA with Ro*M* NPV. Of eight baculovirus DNAs tested, only Ac*M* NPV DNA and Ro*M* NPV DNA could transfect TN-368 cells. The *in vitro* host range of Ac*M* NPV DNA was determined to be the same as Ac*M* NPV extracellular virus. Ac*M* NPV Form I DNA was fourfold more infectious in TN-368 cells than Form II DNA.

Baculoviruses are rod-shaped viruses which contain high molecular weight (58–103 × 10^6 daltons) covalently closed DNA (*10*, *11*, *15*). The potential use of these viruses as cloning vectors and viral pesticides has provided incentive for a more detailed knowledge of the genetics of baculovirus genomes (*12*). In particular, major interest has been placed on the molecular biology of the nuclear polyhedrosis virus of *Autographa californica* (Ac*M* NPV) emphasizing: biology and replication in susceptible insect hosts and in cell culture (*18*, *19*); structural polypeptides (*2*, *16*); and physical mapping of wild isolates and plaque-purified variants (*9*, *12*).

Although cell culture systems are being used to study the replication and *in vitro* host range of these viruses, little is known about the infectivity of baculovirus DNAs. In this report, we have used the calcium phosphate precipitation technique (*5*) to determine the efficiency of transfection of Ac*M* NPV DNA in several invertebrate cell lines, and have conducted preliminary studies to compare the ability of other baculovirus DNAs to infect TN-368 cells.

The continous insect cell lines used in this study were: TN-368 from our laboratory (*18*), *Spodoptera frugiperda* IPLB-SF-21 obtained from Dr. D. L. Knudson (*7*), *Manduca sexta* from Dr. D. B. Stoltz, *Malacosoma disstria* IPRI-Md-108 from S. S. Sohi, and *Lymantria dispar* IPLB-PD-65z from Dr. J. L. Vaughn. All cell lines were maintained and subcultured as previously described (*18*). Ac*M* NPV E2, a plaque-purified isolate that has been physically mapped, was used for this study (*12*). DNAs that were purified from other viruses included *Rachiplusia ou* (Ro*M* NPV) from Dr. C. Y. Kawanishi, *Christoneura fumiferana* (Cf*M* NPV) from Dr. B. M. Arif, a plaque-purified isolate of *Heliothis zea* nonoccluded virus (HZ-1) obtained from Dr. C. Kawanishi (*6*), *Spodoptera frugiperda* granulosis virus (SfGV) and *Heliothis zea* (Hz*S* NPV) from Dr. J. J. Hamm, and from this laboratory the *S* NPV and GV of *Trichoplusia ni*.

Baculovirus polyhedra were recovered from infected larvae and the virus was purified from alkaline-dissolved polyhedra by equilibrium banding of the virus at a density of 1.21–1.25 in sucrose gradients as described by Summers and Smith (*16*). The HZ-1 nonoccluded virus was plaque-purified and subsequently mass propagated in TN-368 cells. At 48 hr postinfected, the HZ-1 virus was purified using the procedure for extracellular virus (ECV) outlined by Smith and Summers (*11*).

Log phase cells from the respective cell lines to be tested for infectivity were handled as described by Volkman *et al.* (*18*) with pertinent details given in Table 1.

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TABLE 1

EXTRACELLULAR VIRUS TITERS FROM AcMNPV AND DNA INFECTED CELLS

Cell line	Titer[a]	
	Virus[b]	DNA[c]
Trichoplusia ni		
TN-368	1.4×10^8	5.9×10^6
Spodoptera frugiperda		
IPLB-SF-21	1.1×10^8	6.7×10^3
Manduca sexta	4.2×10^8	4.5×10^3
Malacosoma disstria		
IPRI-Md-108	8.1×10^6	4.0×10^2
Lymantria dispar		
PD-65z	$1.7 \times 10^{4\,d}$	0[e]

[a] Infectivity of AcMNPV and DNA (PFU/ml at 72 hr postinfection) in permissive and nonpermissive insect cells was measured by titration of ECV produced in these cell lines using a plaque assay with TN-368 cells as indicator (*17*). The results are expressed as the mean value of duplicate assays each performed in triplicate.

[b] Log phase cells from the respective cell lines to be tested were centrifuged at 400 *g* for 10 min. The supernatant was removed and the cells were resuspended in medium without FCS. For each cell line, three disposable tissue culture flasks (25 cm^2) were seeded with 1.0×10^6 cells in 3 ml of medium without FCS. The cells were placed at 28° for 30 min and allowed to attach to the flask. To infect the cells the medium was removed, and 1.0 ml of AcMNPV inoculum containing 5×10^6 PFU (m.o.i. of 5 standardized in TN-368 cells) was added (*18*). The flasks were rocked slowly on a rocker platform for 1 hr to allow the virus to adsorb. After this period, the inoculum was removed and the cells were washed two times with 1.0 ml of fresh medium. Each flask then received 3.0 ml of fresh medium plus gentamicin and fungizone (0.1 mg/ml), and was incubated at 28°. At 72 hr postinfection the supernatant containing ECV was collected centrifuged at 400 *g* to remove cell debris and polyhedra, then back-titrated into TN-368 cells using the plaque assay procedure of Wood (*20*) as modified by Summers *et al.* (*17*).

[c] One microgram of AcMNPV DNA and 15 μg/ml calf thymus DNA were precipitated with $CaCl_2$ and added to each tissue culture plate containing preattached cells as described in Fig. 1. At 72 hr postinfection, the ECV was separated from residual precipitated DNA, intact cells and cellular debris by low speed centrifugation, then titered by plaque assay.

[d] This is not an increase in titer over residual input virus.

[e] No plaques or polyhedra were detected.

DNAs used for most transfection studies were obtained from purified virus preparations following the procedure of Smith and Summers (*11*) as detailed in Fig. 1. To compare the infectivity of Forms I and II (Fig. 2), purified DNA was prepared as described in Fig. 1 except that the proteinase K treatment was for 30 min. The DNA was centrifuged to equilibrium in cesium chloride–ethidium bromide (CsCl–EtBr) gradients and the band with the light density (1.54 g/ml) and that of heavy (1.58 g/ml)

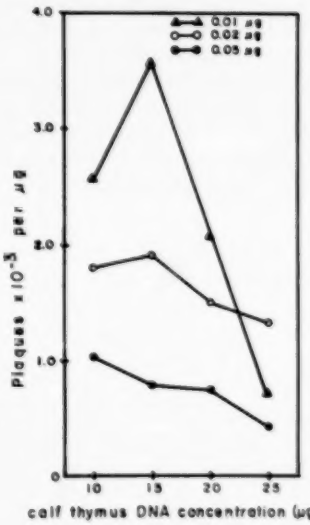

FIG. 1. Effect of calf thymus DNA carrier concentration of the infectivity of AcMNPV E2 DNA. Viral isolates were lysed using 2.0% sodium lauryl sacrosinate (SARC) in 0.1 *M* Tris, 0.01 *M* EDTA, pH 7.5, at 37° for 15 min. Proteinase K (predigested 30 min at 37°) was added at a final concentration of 2.0 mg/ml and incubated for 3 hr at 37°. Each preparation was extracted twice with buffer-saturated phenol and once with chloroform isoamyl alcohol (24:1), then dialyzed extensively against 0.1 × SSC. AcMNPV DNA (0.01 (▲); 0.02 (○); and 0.05 (●) μg/plate) was mixed with increasing amounts of calf thymus DNA (10–25 μg/ml) in HEPES-buffered saline (*5*), pH 7.05. The DNA was precipitated for 30 min using $CaCl_2$ at a final concentration of 120 m*M*, then 0.2 ml was pipetted onto a preattached cell monolayer and absorbed for 1 hr. The precipitate was removed and the plate overlayed with 1.5% agarose, and scored for plaque production by polyhedral plaque assay (*20*).

density were collected dropwise through the bottom of the tubes (*11*). After isoamyl alcohol extraction and extensive dialysis against 0.1 × SSC, 1.0–2.0 μg of DNA from each band was analyzed by sedimentation in neutral sucrose gradients (*15*).

The method used for transfection was essentially that of Graham and van der Eb (*5*). The efficiency of transfection of Ac*M* NPV DNA in TN-368 cells was determined by plaque forming ability of the calcium phosphate–DNA complex. The infectivity of Ac*M* NPV DNA in permissive and nonpermissive insect cell lines was measured as reported by Summers *et al.* (*17*) following the method outlined in Table 1.

Initial attempts to transfect insect cells with baculovirus DNA (5 and 10 μg/ml) without the addition of $CaCl_2$ were successful. However, infectivity (190 PFU/ug) was obtained using $CaCl_2$ as facilitator at a final concentration of 120 m*M* (*5*). A 20% dimethyl sulfoxide boost 4 hr after the addition of precipitated DNA (*1*, *13*) yielded as much as a 2.5-fold increase in PFU/per microgram of DNA, however, this treatment was not routinely used. Addition of calf thymus DNA carrier further increased the infec-

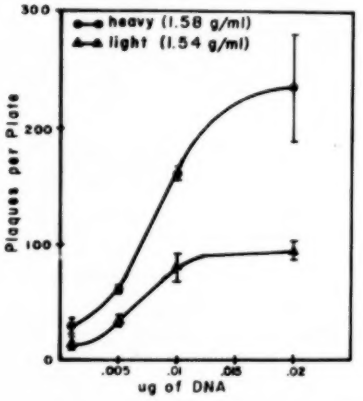

FIG. 2. Comparison of infectivity of Ac*M* NPV E2 light and heavy density bands by transfection. DNA was prepared as outlined in Fig. 1 except proteinase K treatment was for 30 min. The DNA was centrifuged to equilibrium in CsCl–EtBr gradients (mean CsCl density 1.50 g/ml with EtBr at 200 μg/ml) at 45,000 rpm for 24 hr using a SW 60 Ti rotor. The resulting bands were obtained by puncturing the bottom of the tubes and collecting the uv-visible fractions. DNAs were extracted three times with isoamyl alcohol and dialyzed extensively against 0.1 × SSC. Infectivity was measured by the plaque assay procedure (*20*). Band of heavier density (●); and the band of lighter density (▲).

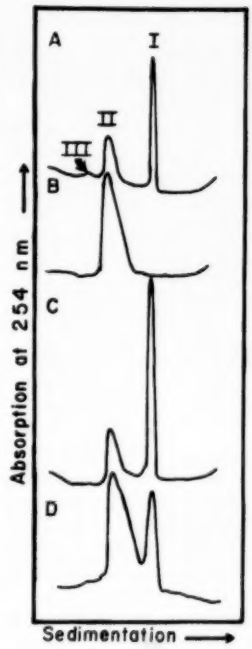

FIG. 3. Neutral sucrose gradient sedimentation of Ac*M* NPV DNA: light and heavy density bands from CsCl–EtBr gradients. Virions were suspended in 0.1 *M* Tris, 0.01 *M* EDTA, pH 7.5, made 0.1 mg/ml proteinase K and 2% SARC, and incubated for 30 min at 37°. DNA from the bands of light and heavy density from CsCl–EtBr gradients were recovered, dialyzed against 0.1 × SSC, then used immediately for transfection. Aliquots of light and heavy density DNA (1–2 μg) bands were carefully layered onto 5–30% (w/v) sucrose gradients constituted in 1.0 m*M* Tris, 0.1 m*M* EDTA, pH 7.5 and centrifuged in an SW 60 Ti rotor at 50,000 rpm for 90 min. Sucrose gradients were fractionated through an ISCO UA-5 ultraviolet analyzer. (A) Ac*M* NPV DNA from the band of heavier density that was frozen and thawed twice to produce Form II DNA, (B) lighter density band, (C) heavier density band, and (D) a mixture of light and heavy density band DNAs.

tivity of Ac*M* NPV DNA to 3600–4100 PFU/ug at an optimal carrier concentration of 15–20 μg calf thymus DNA/ml (Fig. 1). Unless otherwise indicated the standard procedure for transfection was as outlined in Fig. 1 using 20 μg/ml of calf thymus DNA as carrier.

The DNA in the band of heavy density recovered from CsCl–EtBr gradients was found to be more infectious (2.5-fold) than the band of lighter density (Fig. 2). We measured the stability of the DNA recovered from each band by sedimentation studies in neutral sucrose (Fig. 3). The band of lighter density consisted of Form II DNA (Fig. 3B), while about 40% of the DNA in the band of heavier density was converted from Form I to Form II (Fig. 3C) during handling and dialysis.

Ac*M* NPV DNA was compared to ECV for ability to infect permissive and nonpermissive cells (Table 1). The titer of progeny ECV from each cell line was measured 72 hr postinfection. The virus and the DNA produced ECV in all cell lines tested except *Lymantria dispar*. We confirmed these results by observing the presence of polyhedra in the permissive cells at 72 hr postinfection. Contrary to previous reports (*7*) we were unable to observe the production of EVC or polyhedra in *L. dispar* cells that had been infected with Ac*M* NPV EVC. The yield of Ac*M* NPV ECV was significantly higher in DNA infected TN-368 cells as compared to the other three permissive cell lines.

We also tested the infectivity of seven other baculovirus DNAs in TN-368 cells using the standard transfection procedure outlined above. Ro*M* NPV DNA produced 2900 PFU/μg. The DNAs from HZ-1, Hz*S* NPV, Cf*M* NPV, SFGV, Tn*S* NPV, and TnGV as well as the four purified Ac*M* NPV DNA *Sma*-1 fragments isolated from agarose gels (*12*) failed to yield plaques upon transfection.

Ac*M* NPV DNA had the same *in vitro* host range as the virus. However, we observed some interesting differences. The titer of ECV at 72 hr postinfection of *Spopotera frugiperda* cells with Ac*M* NPV DNA (6.7×10^3 PFU/ml) was significantly less than from TN-368 cells (5.9×10^6 PFU/ml). Whereas, after infection with equivalent multiplicities of virus, these two cell lines produced comparable titers ($1.0–1.4 \times 10^8$) of ECV 72 hr postinfection.

Sedimentation analysis of Ac*M* NPV DNA demonstrated the presence of DNA Forms I and II from enveloped nucleocapsids. There was no detectable Form III (linear) DNA found in fresh preparations of Ac*M* NPV DNA or in DNA preparations used for transfection. About 1% of Forms I and II was converted to Form III DNA by a cycle of freeze–thaw (Fig. 3A). Analysis of DNA from the band with heavier density indicated that the covalently closed molecule was unstable. In spite of rigorous attempts to minimize physical or nuclease degradation, at least 40% of the DNA was converted to the relaxed form. The infectivity of the heavy density band was two- to threefold greater than the light density band. Therefore Form I DNA of Ac*M* NPV can be estimated to be fourfold more infectious than Form II DNA. In a similar study with polyoma virus, Form I DNA is shown to be two- to threefold more infectious than Form II (*3*).

In a previous report (*18*) we estimated the infectivity of alkali liberated virus to be 8.4×10^5 PFU/ug of DNA and the infectivity of ECV to be 8.9×10^8 PFU/ug of DNA. Therefore the efficiency of transfection is 2.0% compared to alkali liberated virus and 0.002% compared to ECV.

We were not able to expand the *in vitro* host range of Ac*M* NPV by transfection nor were we able to extend the host range of other baculoviruses to TN-368 cells by this method. Furthermore, HZ-1 DNA (2.5–5.0 μg/ml) did not form plaques in TN-368 cells. In the control experiment the virus does form plaques in these cells (unpublished results and C. Y. Kawanishi, personal communication). This later observation suggests that certain HZ-1 structural proteins may be necessary for the productive infection of TN-368 cells by this virus.

Although we were not able to extend the *in vitro* host range of these baculoviruses by transfection, this technique will prove useful in studies using infectious DNA, such

as marker rescue (*4*, *14*) and for mapping of baculovirus gene products.

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